THE WAX-EXUDING, CUTICULAR PORES OF *APIOMORPHA* RUBSAAMEN (HOMOPTERA, COCCOIDEA): A LIGHT MICROSCOPY AND SCANNING ELECTRON MICROSCOPY STUDY

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Abstract

The cuticular pores of adult females of the gall-forming genus *Apiomorpha* Rubsaamen are all multilocular disc pores that exude only curved filaments of white, powdery wax. The structure, distribution and wax exudation of these disc pores are described and discussed and a comparison is made between the appearance of the pores using light microscopy and scanning electron microscopy.

Introduction

The structure and distribution of wax-exuding pores of the adult female are used in the description and identification of coccid species (e.g. Ferris 1950, 1957; Beardsley 1959; Hoy 1962; McKenzie 1967; Miller 1970; Williams and Kosztarab 1972). Pores of several distinct structural types have been described (e.g. Ferris 1950; McKenzie 1967; Kawai and Tamaki 1967) and a few studies have associated particular pore types with the presence of certain types of wax (Kawai and Tamaki 1967; Tamaki, Yushima and Kawai 1969; Gimpel, Miller and Davidson 1974; Hamon, Lambdin and Kosztarab 1975). This paper is concerned with one type of disc pore and its exudation. Disc pores are classified as trilocular, quadrilocular, quinquelocular or multilocular, depending on the number of openings (loculi) that comprise each pore (Ferris 1950).

The conventional method for examination of cuticular details of coccids requires the preparation of cleared and stained specimens mounted on microscope slides (Kozarzhevskaya 1968). Scanning electron microscopy has also been used in several structural studies on the wax and the wax-exuding pores of coccids (Hashimoto and Kitaoka 1971; Miller and Gimpel 1974; Gimpel *et al.* 1974; Miller, Marsh and Gordon 1975; Miller 1975; Knipscher, Miller and Davidson 1976), but no direct comparison between the appearance of pores using the light microscope and the scanning electron microscope seems to have been published. Light microscopy investigations have mainly concentrated on the histology of the wax-producing glands (Pollister 1937; Lower 1957) or on the structural interpretation of different pore types using only slide-mounted specimens (e.g. Ferris 1950; McKenzie 1967; Kawai and Tamaki 1967).

This paper describes the structure, distribution and wax exudation of the disc pores of the gall-forming genus *Apiomorpha* Rubsaamen and compares the appearance of the pores using light microscopy and scanning electron microscopy.

Materials and methods

Adult females of six species*, *Apiomorpha conica* (Froggatt), *A. munita* (Schrader), *A. ovicola* (Schrader), *A. pharetrata* (Schrader), *A. strombylosa* (Tepper) and one new, undescribed species, were examined with the scanning electron microscope. Fresh specimens were killed in 70% ethanol. Preserved specimens had been stored either in 70% ethanol or in a lactic acid-ethanol mixture (Stroyan 1949). All were collected by the author. Each specimen was cut open three-quarters of the way around the body along the dorso-ventral line, cleared in cold 10% potassium hydroxide for 24 hours, washed gently in distilled water to remove all body contents and placed in a small petri dish with dorsum and venter opened out to lie adjacent, still connected to each other. A coverslip was placed over the preparation to keep the cuticle flat during subsequent dehydration and the specimen was then bathed for one hour in acid alcohol (see method of Williams in Kozarzhevskaya 1968).

The preparation of specimens for both the scanning electron microscope and the light microscope was identical until this stage. For the former, specimens were then dehydrated using ethanol, transferred to absolute amyl acetate via a graded ethanol-amyl acetate series, placed on a 2.8 cm diameter specimen stub and allowed to air-dry. Shrinkage was not a problem since the cuticle of the pore walls appears to be stabilized by sclerotization. Silver dag was used to improve contact between specimen and stub. Specimens were coated with gold for three minutes at 30 mA in a model SC150 Dynavac Sputter Coater and examined in a Cambridge Stereoscan S4-10 S.E.M. at an accelerating voltage of 20 kV. Photomicrographs were taken with Polaroid Type 665 positive/negative film.

For light microscopy, specimens were stained for 1-5 minutes in a 50% acid fuchsin stock solution in water, dehydrated in ethanol, transferred to xylene and mounted in canada balsam on microscope slides (a modification of Williams' method in Kozarzhevskaya 1968). Photomicrographs were prepared using bright field illumination on a Leitz Orthoplan microscope equipped with an Orthomat camera using Copex Pan Rapid film. Specimens were also examined using Heine phase contrast illumination.

Only the pores of the venter, especially the abdominal segments, were intensively studied, although the distribution and characteristics of all body pores were noted. Unless otherwise stated, the structural descriptions refer to ventral pores.

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* Collection data for specimens used in this study:– *A. conica* (Froggatt)–ex *Eucalyptus viminalis* Labill., Cranbourne Botanic Gardens Annexe, Cranbourne, Vic., 27.i.1977; *A. munita* (Schrader)–ex *E. goniocalyx* F. Muell. ex Miq., Mt Granya, *c.* 12 km NE Tallangata, Vic., 29.v.1975; *A. ovicola* (Schrader)–ex *E. camaldulensis* var. *obtusa* Blakely, Maloneys Creek, *c.* 6.5 km N Finke River and *c.* 110 km SW Alice Springs, N.T., 29.v.1977; *A. pharetrata* (Schrader)–ex *E. macrorhyncha* F. Muell. ex Benth., Mt Granya, *c.* 12 km NE Tallangata, Vic., 25.v.1976; *A. strombylosa* (Tepper)–ex *E. polyanthemos* Schauer in Walp., 134 Brackenbury Street, Warrandyte, Vic., 7.v.1977; *Apiomorpha* sp.–ex *E. leptopoda* Benth., Great Eastern Highway, *c.* 80 km E Southern Cross, W.A., 3.iv.1978.

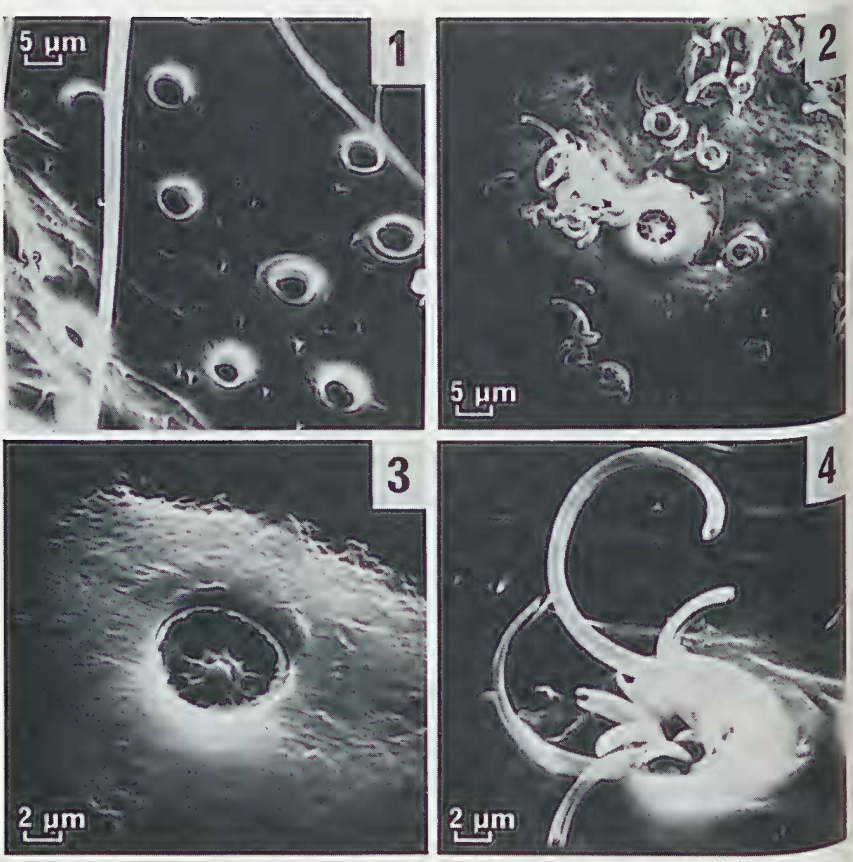
Pore structure and wax exudation

The wax-exuding structures of *Apiomorpha* are all multilocular disc pores, with locular numbers varying from 7 to 19. Nine-locular and 11-locular disc pores are most numerous on the dorsum and venter of *A. ovicola, A. pharetrata, A. strombylosa* and the undescribed species, particularly on the abdominal segments, while 11-locular and 13-locular disc pores occurred most commonly on the specimens of *A. conica* and *A. munita* that were examined. However, certain geographic populations of *A. munita* show a predominance of 7-, 9- and 11-locular disc pores.

Pore diameter, as measured from scanning electron micrographs, ranged from 3.0 μm for a few abdominal pores of *A. conica* (Fig. 1) to 8.0 μm for some pores of *A. ovicola.* The wax-exuding part of each pore is sunken below the rim to varying degrees, depending on the species, and in some species each pore is surrounded by a broad, raised rim that is more sclerotized than the surrounding cuticle (Fig. 2). The disc pores of *A. conica* are especially depressed and the rim is more flange-like than in the other species (Fig. 1). Each disc pore possesses a central, sclerotized, flattened (as in *A. conica*) or, more usually, convex structure (*A. munita, A. ovicola, A. pharetrata, A. strombylosa* and the undescribed species) that is encircled by the loculi (Figs 2, 3). Hence there is no central aperture to the disc pores of *Apiomorpha*, in contrast to the situation in some other coccids (Gimpel *et al.* 1974; Hamon *et al.* 1975). For instance Gimpel *et al.* (1974) state that the multilocular pores of the genus *Ceroplastes* Gray (Coccidae) possess a central, circular loculus.

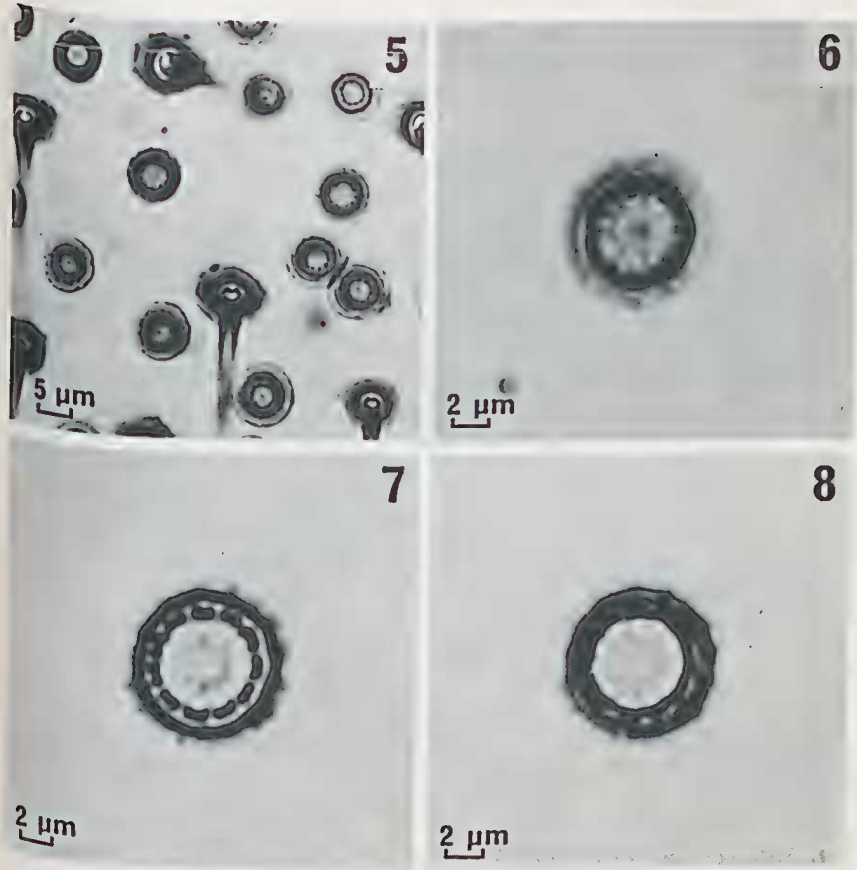
Each locule appears to exude a curved filament of wax (Figs 2, 4) that is trough-like or almost C-shaped in cross-section and about 1 μm in diameter. The structures that are just visible on the outer edge of the loculi of Fig. 3 are the truncate ends of wax filaments. The preparation technique probably dislodged the exposed filaments from most of the specimens examined. Wax was most often observed in specimens prepared from recently-killed individuals, especially those from species that were prolific wax-producers. These wax filaments are white and powdery in living specimens.

In the genus *Ceroplastes* the multilocular pores are mainly confined to the vulvar region and have been associated with the exudation of powdery or granular, white wax (Kawai and Tamaki 1967; Tamaki *et al.* 1969; Gimpel *et al.* 1974). In *Kermes kingi* Cockerell (Kermesidae), filaments of wax were presumed to be exuded by transverse abdominal rows of multilocular pores on the venter of the adult female (Hamon *et al.* 1975). These studies have suggested that powdery wax filaments, apparently exuded from multilocular pores, probably function to prevent eggs from adhering to each other and to the brood chamber and to protect eggs from desiccation. The chemical composition of the powdery wax has not been reported, but glands associated with multilocular disc pores of two coccids have been shown to be multicellular (Pollister 1937; Tamaki *et al.* 1969). The extrusion of wax filaments that is observed in *Apiomorpha* (Fig. 4) provides evidence that the powdery wax is actually exuded by loculi of the multilocular disc pores.



Figs 1-4. Scanning electron micrographs of ventral, abdominal, wax-exuding pores of *Apiomorpha* Rubsaamen: (1) multilocular pores and minute, spine-like processes of *A. conica* (Froggatt); (2) 9-locular pore and wax filaments of *A. ovicola* (Schrader); (3) 11-locular pore of *A. strombylosa* (Tepper); (4) pore of undescribed species in process of exuding wax filaments.

In adult females of *Apiomorpha* the multilocular disc pores generally occur on all body segments of the dorsum and venter, although pores are mostly absent from the ninth abdominal segment and are never present on the anal lobes. Both the surface of the adult female and the walls of the gall chamber are usually coated with white, powdery wax and the presence of this wax at the orifice of the gall, in many species, indicates that the gall houses a live insect. In species where the female has very few pores [e.g. *A. calycina* (Tepper)] a negligible amount of wax is present. Wax secretion does not appear to be closely associated with parturition in *Apiomorpha*, but probably prevents the female from becoming covered with its own honeydew excreta and may protect the female against



Figs 5-8. Light photomicrographs of ventral, abdominal, wax-exuding pores of *Apiomorpha* Rubsaamen: (5) *A. conica* (Froggatt)—an area similar to that of Fig. 1, but spine-like and hair-like setae present; (6) 7-locular pore of *A. ovicola* (Schrader); (7, 8) same 11-locular pore of *A. ovicola* at two different focal planes.

desiccation. The latter suggestion is supported by the observation that species in which the female produces very little wax possess galls with minute orifices, which would serve to restrict water loss from the gall cavity. The former function has been discussed by Broadbent (1951) in relation to gall-living aphids and it is notable that in *Apiomorpha* some wax-exuding pores are always present on the posterior abdominal segments, which are most likely to come into contact with excreta, while pores may be reduced in number or absent from the anterior of the body. In some other coccids (Williams 1978), instances of reduction in number or absence of the wax-exuding pores and ducts have been shown to be associated with the myrmecophilous habit. This possibly suggests that the wax secretion of at least some coccids functions chiefly to prevent contamination

from the coccid's own honeydew rather than to prevent desiccation. A very close association with ants would reduce the need for a waxy covering because the ants would quickly remove any honeydew that was produced.

Pore structure

Light microscopy compared with scanning electron microscopy

Figs 5-8 are light photomicrographs, taken with bright field illumination, of well-stained disc pores that were observed on recently-moulted adult females of *A. conica* and *A. ovicola.* In mature specimens the general body cuticle is thicker and differential staining is difficult to achieve. The photographic quality of stained mature specimens and unstained material is poor and the use of phase contrast illumination does not significantly improve the image.

Light photomicrographs show the following inadequacies: the structure of disc pores is difficult to determine due to poor resolution (Figs 5, 6) and the image has a variable appearance due to limitations of focal depth (compare Figs 7 and 8). Figs 1 and 5, which are of similar areas of the abdominal cuticle of *A. conica* and at comparable magnification, demonstrate the improved resolution and greater depth of focus that is attainable with the scanning electron microscope. From the light photomicrograph (Fig. 5) the sunken nature of the disc pores is not apparent and the minute, spine-like, non-cellular processes of the body cuticle, that are seen clearly in Fig. 1, only appear as faint, darkish spots.

Disc pores of different locular number (compare Fig. 6 with Figs 7 and 8) may display apparent variation in structure that cannot entirely be attributed to differences in the focal plane. This variation may be due to disparity in the passage of light through loculi of different size.

The use of both the light microscope and the scanning electron microscope provides an integrated description of the wax-exuding pores. While light microscopy is essential for determining the distribution of disc pores of different locular number, a knowledge of pore ultrastructure allows the accurate description of pore structure and the interpretation of the conventional light microscope image. The value of the scanning electron microscope in determining the complicated structure of wax-exuding pores has been recognised by Tamaki *et al.* (1969), Miller and Gimpel (1974) and Miller *et al.* (1975). Furthermore Miller (1975) has suggested that pore ultrastructural differences may be useful for separating species.

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References

Beardsley, J. W., 1959. On the taxonomy of pineapple mealybugs in Hawaii, with a description of a previously unnamed species (Homoptera: Pseudococcidae). *Proc. Hawaii. ent. Soc.* 17(1): 27-37.

Broadbent, L., 1951. Aphid excretion. *Proc. R. ent. Soc. Lond.* (A)26: 97-103.

Ferris, G. F., 1950; *Atlas of the scale insects of North America* (5)5; *The Pseudococcidae (Part I)*. Stanford University Press, California. 278 pp.

Ferris, G. F., 1957. Notes on some little known genera of the Coccoidea (Homoptera). *Microentomology* 22(3): 59-79.

Gimpel, W. F. Jr, Miller, D. R. and Davidson, J. A., 1974. A systematic revision of the wax scales, genus *Ceroplastes*, in the United States (Homoptera; Coccoidea; Coccidae). *Md Agric. Exp. Sta. Misc. Publ.* 841: 1-85.

Hamon, A. B., Lambdin, P. L. and Kosztarab, M., 1975. Eggs and wax secretion of *Kermes kingi*. *Ann. ent. Soc. Am.* 68(6): 1077-1078.

Hashimoto, A. and Kitaoka, S., 1971. Scanning electron microscopic observation of the waxy substances secreted by some scale insects. *Jap. J. appl. Ent. Zool.* 15: 76-86.

Hoy, J. M., 1962. Eriococcidae (Homoptera: Coccoidea) of New Zealand. *Bull. N.Z. Dep. scient. ind. Res.* 146: 1-219.

Kawai, S. and Tamaki, Y., 1967. Morphology of *Ceroplastes pseudoceriferus* Green with special reference to the wax secretion. *Appl. Ent. Zool.* 2(3): 133-146.

Knipscher, R. C., Miller, D. R. and Davidson, J. A., 1976. Biosystematics of *Chionaspis nyssae* Comstock, with evidence supporting leaf and bark dimorphism of the scale. *Melanderia* 25: 1-30.

Kozarzhevskaya, E. F., 1968. Technique of preparing slides for coccid (Homoptera, Coccoidea) determination. *Ent. Rev., Wash.* 47(1): 146-149.

Lower, H. F., 1957. A comparative study of the cuticular structure of three female mealy bugs (Homoptera: Pseudococcidae). *Biol. Bull. mar. biol. Lab., Woods Hole* 113(1): 141-159.

McKenzie, H. L., 1967. *Mealybugs of California with taxonomy, biology, and control of North American species (Homoptera: Coccoidea: Pseudococcidae)*. University of California Press, Berkeley.

Miller, D. R., 1970. A new genus and species of scale insect from Tasmania (Homoptera: Eriococcidae). *J. Aust. ent. Soc.* 9: 157-159.

Miller, D. R., 1975. A revision of the genus *Heterococcus* Ferris with a diagnosis of *Brevennia* Goux (Homoptera: Coccoidea: Pseudococcidae). *Tech. Bull. U.S. Dep. Agric.* No. 1497: 1-61.

Miller, D. R. and Gimpel, W. F. Jr, 1974. Scale insects. *Electron Microscope Central Facility Newsl. Univ. Md* 2: 5.

Miller, D. R., Marsh, P. M. and Gordon, R. D., 1975. The scanning electron microscope as a tool for studies in insect taxonomy. *Electron Microscope Central Facility Newsl. Univ. Md* 3: 12.

Pollister, P. F., 1937. The structure and development of wax glands of *Pseudococcus maritimus* (Homoptera, Coccidae). *Qd J. microsc. Sci.* 80: 127-152.

Stroyan, H. L. G., 1949. Aphid technique for advisory entomologists. *Min. Agric. Fish. Conf. of Advisory Entomologists, Occasional Notes* No. 8: 1-9.

Tamaki, Y., Yushima, T. and Kawai, S., 1969. Wax secretion in a scale insect, *Ceroplastes pseudoceriferus* Green (Homoptera: Coccidae). *Appl. Ent. Zool.* 4(3): 126-134.

Williams, D. J., 1978. The anomalous ant-attended mealybugs (Homoptera: Pseudococcidae) of south-east Asia. *Bull. Br. Mus. nat. Hist.* (Ent.)37(1): 1-72.

Williams, M. L. and Kosztarab, M., 1972. Morphology and systematics of the Coccidae of Virginia with notes on their biology (Homoptera: Coccoidea). *Va Polytech. Inst. State Univ. Res. Div. Bull.* 74: 1-215.